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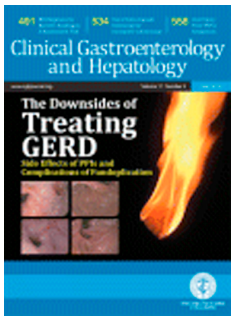

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Efficacy and Safety of Mycophenolate Mofetil and Tacrolimus as Second-line Therapy for Patients with Autoimmune Hepatitis

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ABSTRACT

Background: Predniso(lo)ne, alone or in combination with azathioprine, is the standard of care (SOC) therapy for autoimmune hepatitis (AIH). However, the SOC therapy is poorly tolerated or does not control disease activity in up to 20% of patients. We assessed the efficacy of mycophenolate mofetil (MMF) and tacrolimus as second-line therapy for patients with AIH.

Patients and methods: We performed a retrospective study of data (from 19 centres in Europe, the United States, Canada, and China) from 201 patients with AIH who received second-line therapy (121 received MMF and 80 received tacrolimus), for a median of 62 months (range, 6–190 months). Patients were categorized according to their response to SOC. Patients in group 1 (n=108) had a complete response to the SOC, but were switched to second line therapy due to side effects of predniso(lo)ne or azathioprine, whereas patients in group 2 (n=93) had not responded to SOC.

Results: There was no significant difference in the proportion of patients with a complete response to MMF (69.4%) vs tacrolimus (72.5%) (P=.639). In group 1, MMF and tacrolimus maintained a biochemical remission in 91.9% and 94.1% of patients, respectively (P=.682). Significantly more group 2 patients given tacrolimus compared to MMF had a complete response (56.5 % vs. 34%, P=.029) There were similar proportions of liver-related deaths or liver transplantation among patients given MMF (13.2%) vs tacrolimus (10.3%) (log-rank, P=.472). Ten patients receiving MMF (8.3%) and 10 patients receiving tacrolimus (12.5%) developed side effects that required therapy withdrawal.

Conclusions: Long-term therapy with MMF or tacrolimus was generally well tolerated by patients with AIH. The agents were equally effective in previous complete responders who did not tolerate SOC therapy. Tacrolimus led to a complete response in a greater proportion of previous non-responder patients compared to MMF.

Key words: autoimmune liver disease, simplified criteria, liver failure, liver transplantation.

INTRODUCTION

Autoimmune hepatitis (AIH) is an immune-mediated liver disorder characterized by the presence of circulating autoantibodies and hypergammaglobulinemia with liver histology showing interface hepatitis [1]. AIH can progress to cirrhosis, liver failure and death if untreated [2].

Corticosteroids, alone or in combination with azathioprine (AZA), remain the standard initial treatment of AIH. This therapy is effective in controlling inflammatory activity, reversing or preventing fibrosis progression and prolonging survival in the majority of patients [1-2]. However, up to 20% of patients do not respond, or are intolerant to standard treatment. There are reports of alternative immunosuppressive drug therapies including cyclosporine, methotrexate, 6-mercaptopurine, rituximab, everolimus, mycophenolate mofetil (MMF), tacrolimus, and infliximab, but there is no established rescue therapy in AIH [3-5].

Different studies using MMF and tacrolimus as initial or rescue therapy have reported variable success rates. While some studies report that MMF is effective in non-responders and in patients that are intolerant to standard therapy, other studies suggest that MMF is an alternative only for AZA-intolerant patients [6-12]. Tacrolimus has also been successfully used in AIH patients who failed to respond to standard treatment [13-16]. The evidence regarding the efficacy and safety of these agents however is based on case series of limited size with short durations of follow up. No study has compared the efficacy of MMF to that of tacrolimus. These limitations preclude formally recommending MMF and/or tacrolimus for patients failing standard therapy.

AIH is a rare disease and few patients are considered for second line therapy. To overcome these limitations and to add to our current knowledge, we conducted a large international multi-centre study to retrospectively evaluate the efficacy and safety of MMF and tacrolimus in AIH patients who were non-responsive or intolerant to standard immunosuppressive therapy.

PATIENTS AND METHODS

Study design

We collected data from patients with an established AIH diagnosis from 19 centers across Europe, USA, Canada and China. AIH was diagnosed based on a combination of autoimmune serology, serum gamma globulin or immune globulin G (IgG) levels and compatible liver biopsy findings [17]. Overlaps of AIH with primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) were classified according to suggested international guidelines [18]. All patients who were treated with second line agents were identified. Patients with

insufficient information or non-compliance to therapy, those presenting with acute severe AIH as well as patients diagnosed with overlapping PBC or PSC were excluded. A flow chart for patient inclusion is presented in Figure 1.

Baseline and follow-up data

Collected patient data included sex, age and laboratory parameters according to Table 1. We recorded data on standard therapy (initial doses, therapy duration and response to treatment) and reasons for switching to second line therapy. Local pathologists in the participating centers evaluated liver biopsies; data from their reports were used in the study. Fibrosis was classified according to the METAVIR scoring system [19].

Stratification based on reason for second line therapy

Patients were divided into two groups depending on the reason for switching therapy to second line therapy. Group 1 patients had a complete response to standard therapy, but were switched to second line therapy due to steroid or AZA side effects. Group 2 patients had no response to standard therapy.

In this study, a complete biochemical response was defined as normalization of serum aminotransferase and IgG levels at any time within six months after starting therapy. Anything less than a complete response was considered a non-response [1].

Statistical analysis

Visual (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) were used to determine the normality of continuous variables. Non-continuous variables were expressed as median (minimum-maximum). The χ 2 test, where appropriate, was used to compare the frequencies in different groups. The Wilcoxon signed-rank test was used for comparison of initial and final doses of MMF, tacrolimus and corticosteroid therapy. The Kaplan–Meier method was used to calculate mortality, from the time of second-line therapy to liver-related death and/or need for liver transplantation, using the log-rank test. SPSS software version 22 (SPSS, Chicago,IL,USA) and MedCalc version 14 (MedCalc, Ghent, Belgium) were used to perform statistical analysis and $p<0.05$ was considered statistically significant.

RESULTS

Characteristics of the patient population

The medical records of 2260 patients with AIH were evaluated. Among 302 identified AIH patients treated with second line agents, 171 received MMF, 114 tacrolimus, 12 cyclosporine, 2 everolimus and one patient each received cyclophosphamide, rituximab or methotrexate. The final study group included 201 AIH patients, 121 received MMF and 80 tacrolimus

(figure 1). The number of cases from each participating center is presented in supplementary table S1.

All patients initially received standard of care (SOC) therapy (predniso(lo)ne 20–60, mg/day alone or in combination with AZA, 50–150 mg/day), except for eight patients who received budesonide in combination with AZA as initial therapy. AZA was stopped in all but two patients when MMF or tacrolimus was initiated. Patients received MMF and tacrolimus at doses of 0.5-2.0 g/day and 1-8 mg/day, respectively. The initial and final doses of MMF and tacrolimus were decided by the treating physician according to biochemical and clinical response. A combination of MMF and tacrolimus or a switch between these agents was considered for patients with a suboptimal response or drug side effects.

The reasons for switching from SOC therapy to second line therapy are presented in Table 2. In the stratification based on response to SOC therapy, 108 (53.7%) were stratified to Group 1 (intolerant to SOC), and 93 (46.3%) to Group 2 (non-responders to SOC). Among patients treated with MMF, 74 (61.2%) were in group 1 and 47 (38.8 %) in group 2. In patients treated with tacrolimus, 34 (42.5 %) were in group 1 and 46 (57.5 %) in group 2 (Table 3).

Response to second-line therapy

The efficacy of MMF and tacrolimus in patients with AIH is presented in Table 3. Overall, the complete response rates were similar between MMF and tacrolimus treated patients (69.4% vs 72.5%, p=0.639). MMF and tacrolimus maintained biochemical response in 91.9% and 94.1% of patients in Group 1 (p=0.682). Significantly more group 2 patients given tacrolimus compared to MMF had a complete response (56.5% vs. 34%, p=0.029). The rates of complete response were significantly lower in group 2 than in group 1, both for patients treated with MMF and for patients treated with tacrolimus, p<0.001 (figure 2).

In responders to second line therapy, the median initial and follow up doses for MMF were 1500 (500-2000) and 1000 (0-2000) mg/day, and for tacrolimus 4 (1-8) and 3 (0-6) mg/day (p<0.001 for both). After initiation of MMF, the median steroid dose was decreased from 10 (2.5-22.5) to 5 (0-10) mg/day, and after initiation of tacrolimus from 10 (5-50) to 5 (0-10) mg/day (p<0.001 for both). During maintenance therapy, the steroid therapy was completely withdrawn in 26 patients treated with MMF and in 20 patients treated with tacrolimus.

Management of suboptimal response

Seven non-responders to MMF showed a complete response after switching to tacrolimus. A combination of MMF and tacrolimus was used in eight suboptimal responders to either agent. This resulted in a complete response in six patients.

Second line withdrawal and side effects

Withdrawal of second line therapy was attempted in 14 of patients after long-term remission. Six patients (4 MMF, 2 tacrolimus) maintained biochemical remission during follow up but eight relapsed and were successfully re-treated with the previous regime.

Side effects that required drug discontinuation were seen in 8.3 % (10/121) of MMF treated patients and in 12.5 % (10/80) of tacrolimus treated patients (p=0.326). MMF was stopped due to leukopenia (n=6), gastrointestinal side effects (n=3) and headache (n=1). MMF was switched to standard therapy (n=4) or to tacrolimus (n=5) and one patient declined further therapy. Moreover, MMF had to be discontinued due to pregnancy in two patients and due to lymphoproliferative disorder in one. Tacrolimus was stopped due to neurological side effects (n=4), hypertension and generalized edema (n=2), gastrointestinal side effects (n=2), hair loss (n=1) and renal failure (n=1). Tacrolimus was converted to standard therapy (n=5), to MMF (n=4) or to everolimus (n=1).

Follow-up duration and outcome

The median follow-up time of 62 (6-190) months was similar for patients treated with MMF and tacrolimus, 45 (6-169) vs. 73 (7–190) months, respectively (p=0.116). The 5 and 10 year follow up rates were 46.5% (53/114) and 22.8% (26/114) for MMF, 59.7% (52/87) and 16.1% (14/87) for tacrolimus.

Liver biopsy was performed in 32 patients before second line therapy was initiated and in 24 of these was repeated after a median 38 months (range 24–78) of biochemical remission. Fibrosis progression was observed in 20% (2/10) and 21.4% (3/14) of patients treated with MMF and tacrolimus, respectively (p=0.932). Eight of 32 patients had stage IV fibrosis before second-line therapy. In four of these eight patients, fibrosis remained stable or decreased while four progressed to liver failure. Five patients died from non-liver related causes, eight patients died from liver related causes and 16 patients underwent liver transplantation during follow up. At the time of writing this paper, 10 patients (8 MMF, 2 tacrolimus) were on a liver transplant waiting list. The rates of liver related death or transplantation were similar in the MMF (13.2%, n=15) and tacrolimus (10.3%, n=9) groups (log rank p=0.472, Figure 3).

Discussion

For the significant number of AIH patients that do not tolerate or have a suboptimal response to standard of care therapy, the future holds a risk for cirrhosis, liver failure, liver transplantation or death [20-22]. Additional treatment options beyond standard therapy with steroids and azathioprine are therefore needed. Over the years, several second line options

have been evaluated but reports have been limited to small case series. This study represents the largest cohort of patients exposed to alternative immunosuppression for the management of AIH. Derived from many treatment centers across Europe, China and North America, it represents a real-world experience of both MMF and tacrolimus in AIH.

In earlier studies [6, 7, 23], MMF induced or maintained biochemical remission in 43-88% of AZA-intolerant patients. Unlike these studies, we did not consider progression to liver failure or stopping MMF due to side effects to be the definition of treatment failure, if the patient continued to be in biochemical remission. This difference in definition criteria may contribute to the higher success rate of MMF in our study group.

Existing data regarding the efficacy of MMF in non-responders to SOC therapy are inconclusive. Some reports found MMF effective, whereas other studies reported complete response rates below 25% in non-responders to SOC therapy [6-10]. Our study results, with a 34 % success rate of MMF in non-responders to SOC therapy, are consistent with the latter reports.

Multiple small observational reports have evaluated tacrolimus as second line therapy in AIH. In three studies, tacrolimus promoted or maintained remission in 93% (31/33) of treated patients [13-15]. In another study, tacrolimus induced biochemical remission in 77% (7/9) of acute AIH [24]. More recently, Ni Than et al reported that 52% (9/17) of AIH patients responded to tacrolimus [25]. We found a 56.5% (26/46) complete response rate to tacrolimus in patients failing SOC therapy. Collectively, our and earlier results suggest that tacrolimus may be superior to MMF as an alternative therapy in patients with non-response to standard therapy.

In our study, seven patients entered remission after switching from MMF to tacrolimus. A combination of MMF and tacrolimus induced a biochemical response in six patients, after an insufficient response to single therapy with either agent alone. Recently, Weiler et al. reported successful rescue treatment with infliximab in 11 AIH patients, of whom a majority failed to respond to MMF and/or tacrolimus [4]. These results suggest that a significant proportion of AIH patients still need alternative treatment strategies. Molecular interventions that block multiple and different pathways or that strengthen immune tolerance may provide paths forward in the treatment of AIH. There is a risk for an increased frequency of drug-induced complications with combination therapy. Balancing the potential treatment related side effects from over-immunosuppression may prove to be a challenge.

We particularly focused on severe drug related side effects of MMF or tacrolimus that resulted in therapy withdrawal. Therefore, information about minor side effects that were

tolerated or resolved with dose adjustments were not collected and analyzed in detail. The participating centers reported no cases of skin malignancies and only one case with lymphoproliferative disorder during MMF therapy. However, the case data forms did not specifically ask for information on malignancies. In two patients MMF was discontinued during pregnancy. The teratogenic potential of MMF should be carefully explained to all patients of childbearing age. The retrospective nature of our study may overestimate the safety profile of MMF or tacrolimus. Considering the long follow-up and the available collected data, we can conclude that both agents appear to be safe alternatives for long-term use in AIH.

A complete biochemical response, the prevention of fibrosis progression and the permanent withdrawal of immunosuppression are desirable objectives in AIH [2]. Of note, 21% of patients who were treated with MMF/tacrolimus had fibrosis progression in follow-up biopsies despite maintaining long-term complete biochemical response. This finding is in line with a recent large study on AIH [21]. Successful long-term withdrawal of MMF or tacrolimus was achieved in six of our patients. Since few alternative therapies are available if MMF and/or tacrolimus fail to control disease activity, we cannot recommend routinely attempting discontinuation of second line agents.

Overall, the frequency of liver-related death/transplantation was not different, despite a higher biochemical response rate in the tacrolimus groups. This may be due to some of the MMF non-responders progressing to liver failure with the complication successfully being managed by switching to or combining therapy with tacrolimus.

Second line agents are more expensive than standard immunosuppression and cost-effectiveness may be an important issue in patients treated with MMF or tacrolimus [22]. Predniso(lo)ne, at daily doses exceeding 10 mg for more than two years is associated with several side effects including osteoporosis, bone fractures, diabetes, hypertension, obesity and psychiatric suffering [1]. Suboptimal AIH therapy is also expensive. Progression to liver failure resulting in experimental medication, morbidity, liver transplantation and other health care efforts comes at a price. In our study, MMF and tacrolimus were tapered to minimal effective doses per biochemical response. Steroid therapy was also possible to reduce to low doses and that undoubtedly minimized its side effects. AIH is a lifelong condition for most patients. Beyond the market price of drugs, all these factors have to be balanced into the equation when cost-effectiveness is discussed.

Our study has all the limitations of a retrospective study. Potentially relevant baseline genetic, serologic and histological features that might affect the therapeutic response were not fully

available for all patents. This limitation precludes identifying independent predictors of the response to therapy with MMF and tacrolimus. Indications, drug doses and types of second line therapy were decided on in a non-standardized way. The initial doses of standard therapy and steroid tapering protocols were not identical among physicians. Also, some patients were initially treated by the referring center. All these factors may lead to a bias in our results. However, only expert centers participated in the study and we are confident that the management of these patients was of high standard according to international guidelines. Our study reflects “real life” experiences and we hope that it will be helpful to clinicians in the selection of an appropriate second line therapy.

In conclusion, the results of this retrospective and non-comparative study suggest that MMF and tacrolimus are safe alternative agents with durable immunosuppressive effects in the treatment of AIH in a significant proportion of patients. MMF and tacrolimus are equally capable of inducing or maintaining remission in responders to standard of care medication, but tacrolimus performed better than MMF for patients previously failing to standard therapy. However, approximately one third of our patients showed a suboptimal response to second line therapy and some progressed into liver failure. This emphasizes the need for additional novel alternative therapies.

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Table 1. General features of patients at time of diagnosis and before second line therapy

Characteristics of the study population at the time of AIH diagnosis

	Overall n=201	MMF n=121	Tacrolimus n=80
Gender (Female), n (%)	156(77.4)	96 (79.3)	60 (75)
Age (years)	35 (7-76)	41 (7-76)	31 (10-67)
ALT x UNL	15 (1.8-87.0)	15.5 (1.8-87.0)	12.3 (1.8-49.0)
AST x UNL	13.3 (1.8-92.3)	13.8 (1.8-92.3)	12.0 (2.1-43.9)
ALP x UNL	1.1 (0.2-6.5)	1.3 (0.3-6.5)	0.8 (0.2-3.5)
Bilirubin (x UNL)	2.3 (0.3-15.5)	2.8 (0.3-13.5)	1.9 (0.3-15.5)
IgG x UNL	1.6 (0.5-4.1)	1.4 (0.6-3.8)	1.7 (0.5-4.1)
ANA, n (%)*	127 (68.6)	80(70.8)	47 (65.3)
SMA, n (%) **	120 (65.6)	70 (61.4)	50 (72.5)
LKM, n (%) ***	4(2.3)	3(3.0)	1(1.4)
Fibrosis scores (III-IV), n (%) ****	95 (54.3)	57 (54.8)	38 (53.5)
Initial steroid dose, mg/d	30 (20-60)	30 (20-60)	30 (20-60)
Group 1	30 (20-60)	30 (20-60)	30 (20-60)
Group 2	30 (25-60)	30 (25-60)	30 (30-60)

Characteristics of the study population before second-line therapy

	Overall	MMF	Tacrolimus
Time to second line (months)	12 (2-256)	12(2-256)	10 (3-182)
ALT x UNL	0.9 (0.3-39.2)	0.9(0.3-39.2)	2.1 (0.4-29.6)
AST x UNL	1.0 (0.4-31.5)	1.0(0.4-31.5)	2.0 (0.5-25.5)
ALP x UNL	0.9 (0.3-5.2)	0.9(0.3-5.2)	0.8 (0.4-2.7)
Bilirubin xUNL	0.9 (0.2-11.2)	0.9(0.3-9.9)	0.9 (0.2-11.2)
IgG x UNL	1.0 (0.4-2.1)	0.9 (0.4-2.1)	1.1 (0.4-1.9)
Fibrosis scores (III-IV), n (%) *****	22 (68.8)	12 (75.0)	10 (62.5)

*ANA, was available in 185; **SMA, in 183; ***LKM, in 170; ****liver biopsy, in 175; ***** Biopsy before second line, in 32. Group 1, patients with complete response to standard therapy; Group 2, patients with no response to standard therapy.

Table 2. Reasons for switching therapy to MMF or tacrolimus

	Overall n=201	MMF n=121	Tacrolimus n=80
AZA intolerance, n (%)	78 (38.8)	56 (46.3)	22 (27.5)
Steroid side effects n (%)	30 (14.9)	18 (14.9)	12 (15.0)
Non response to standard therapy, n (%)	93(46.3)	47(38.8)	46 (57.5)

Table 3. Efficacy of MMF and tacrolimus in patients with autoimmune hepatitis			
	MMF (n=121)	Tacrolimus (n=80)	*P* value
Response Complete (all)	84 (69.4%)	58 (72.5%)	0.639
Group I (n=108)	n=74	n=34	
Complete Response	68 (91.9%)	32 (94.1%)	0.682
Group II (n=93)	n=47	n=46	
Complete Response	16 (34.0%)	26 (56.5%)	0.029

Supplementary table S1

The participating centers in alphabetical order and the number of contributed patients

Participating centre	**Number of cases**
Brighton and Sussex University, UK	6
CHU Reims, France	9
Ege University, Turkey	5
Hacettepe University, Turkey	7
Karolinska Institutet, Sweden	45
Kings College Hospital, UK	28
Leipzig University, Germany	32
Mount Sinai Medical Center, USA	8
Numune Education Hospital, Turkey	8
Shanghai Jiao Tong University, China	11
Skane University Hospital, Sweden	8
Umeå University, Sweden	7
University of Alberta, Canada	28
University of Barcelona, Spain	2
University of Bologna, Italy	10
University of British Columbia, Canada	23
University of California San Francisco, USA	4
University of Copenhagen, Denmark	57
University of Gothenburg, Sweden	4
In total 19 centers	***In total 302 patients***

Figure legends:

Figure 1: Study flow chart for patient inclusion.

Figure 2: Therapy response rates for patients treated with MMF and tacrolimus. Complete response rates were significantly decreased through group1 to group 2. ($p<0.001$ for both).

Figure 3: Cumulative survival from liver-related death and transplantation in patients treated with MMF and tacrolimus (log-rank, $p=0.472$).